Center for Protein Folding Machinery

http://proteinfoldingcenter.org

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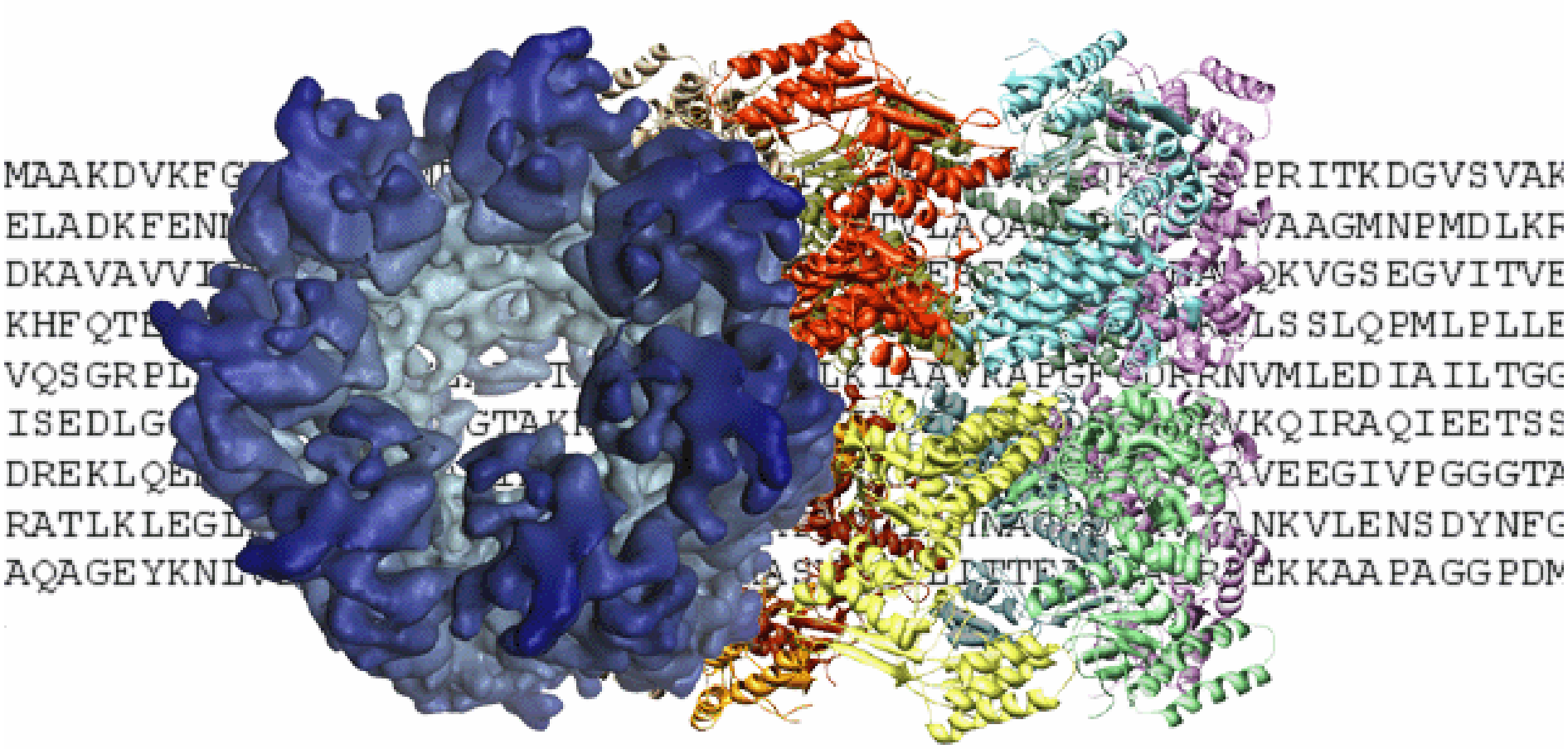

Virtual Center Investigators

Biophysicists/ Chemists

Wah Chiu, Baylor College of Med.
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W. E. Moerner, Stanford U
Steven Chu, Lawrence Berkeley Lab
Paul Adams, Lawrence Berkeley Lab

Biologists/Clinicians

Judith Frydman, Stanford U
Jonathan King, MIT
Huda Zoghbi, Baylor College of Med.
Eric Jonasch, UT MD Anderson Cancer Center

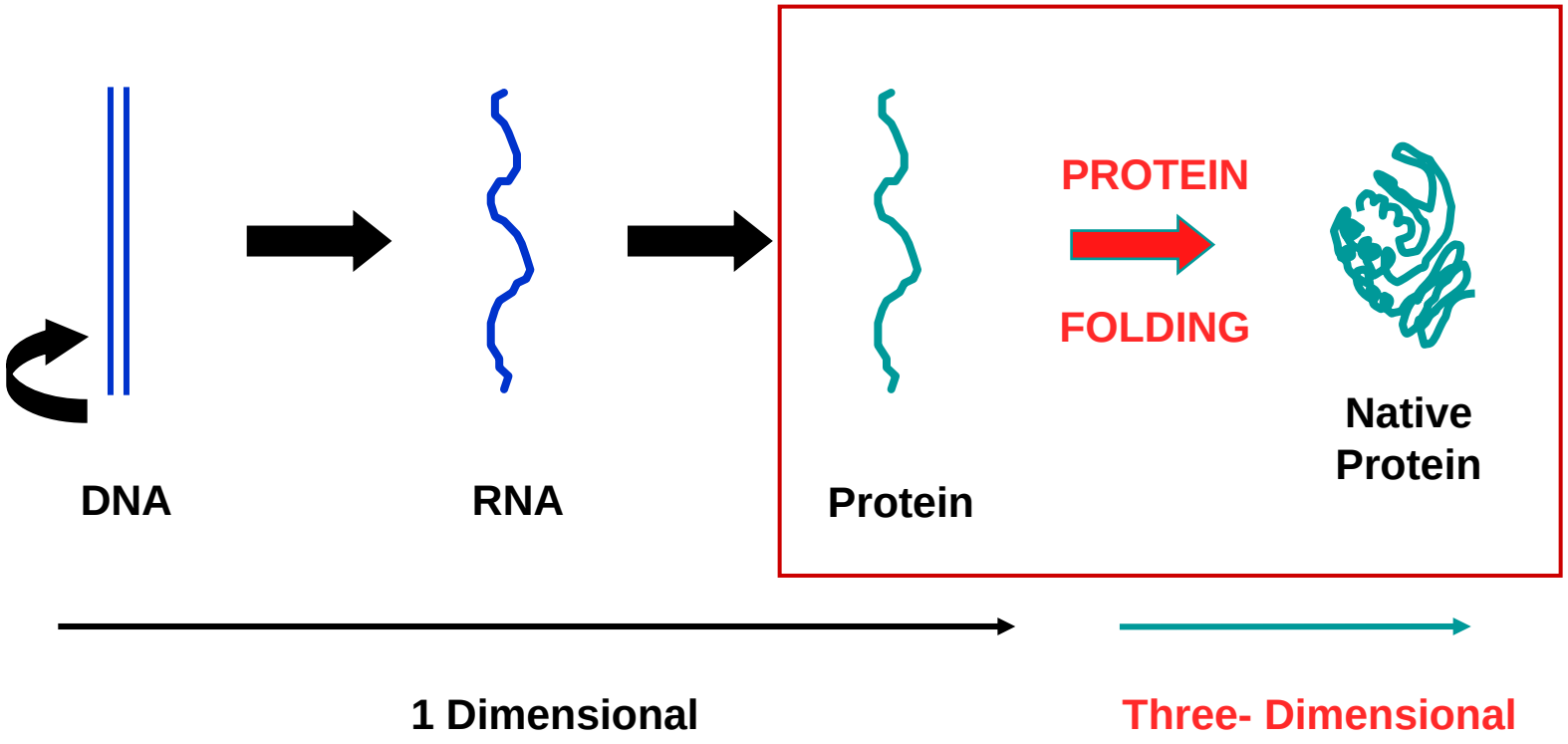
Computational Biologists

Michael Levitt, Stanford U
Vijay Pande, Stanford U
Andrej Sali, UCSF
Tanja Kortemme, UCSF

Engineers

David Gossard, MIT
Scott Delp, Stanford U

Protein Folding is a Key Step in Gene Expression

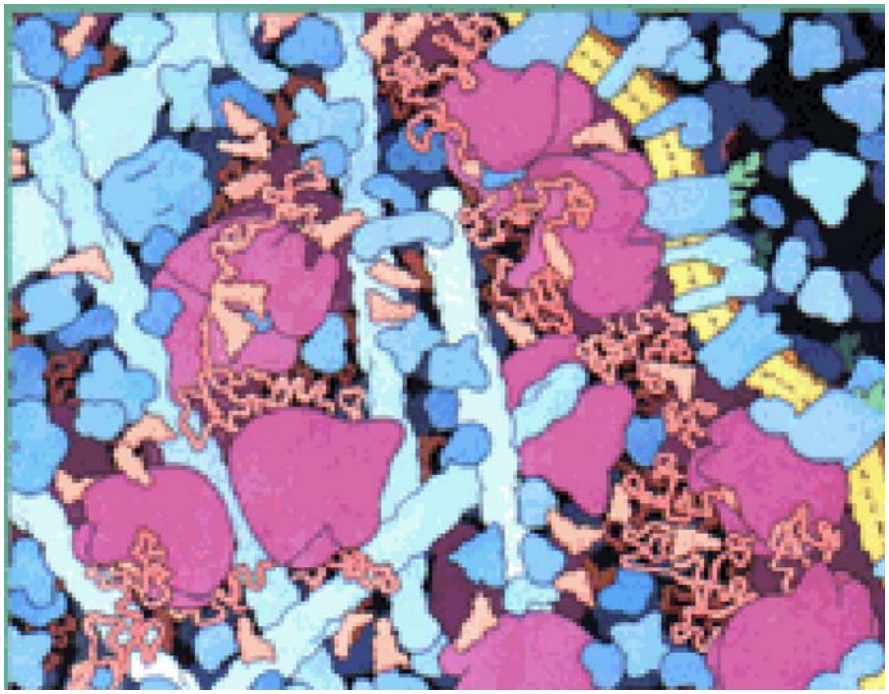


Spontaneous Refolding is only efficient

- for small proteins (less than ~30K)
- very dilute solutions
- low temperatures

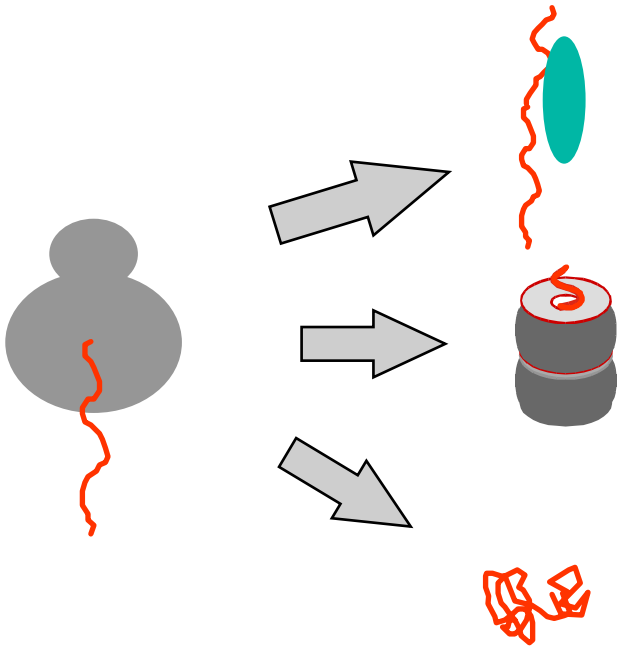
HOWEVER IN VIVO:

Proteins can be very large
Cytosol is Highly Crowded (200-300 mg/ml)
Temperatures >25 °C



How Does the Cell Deal with These Conditions???

Chaperone-mediated Folding in the Cell



Hsp70
~ 20%

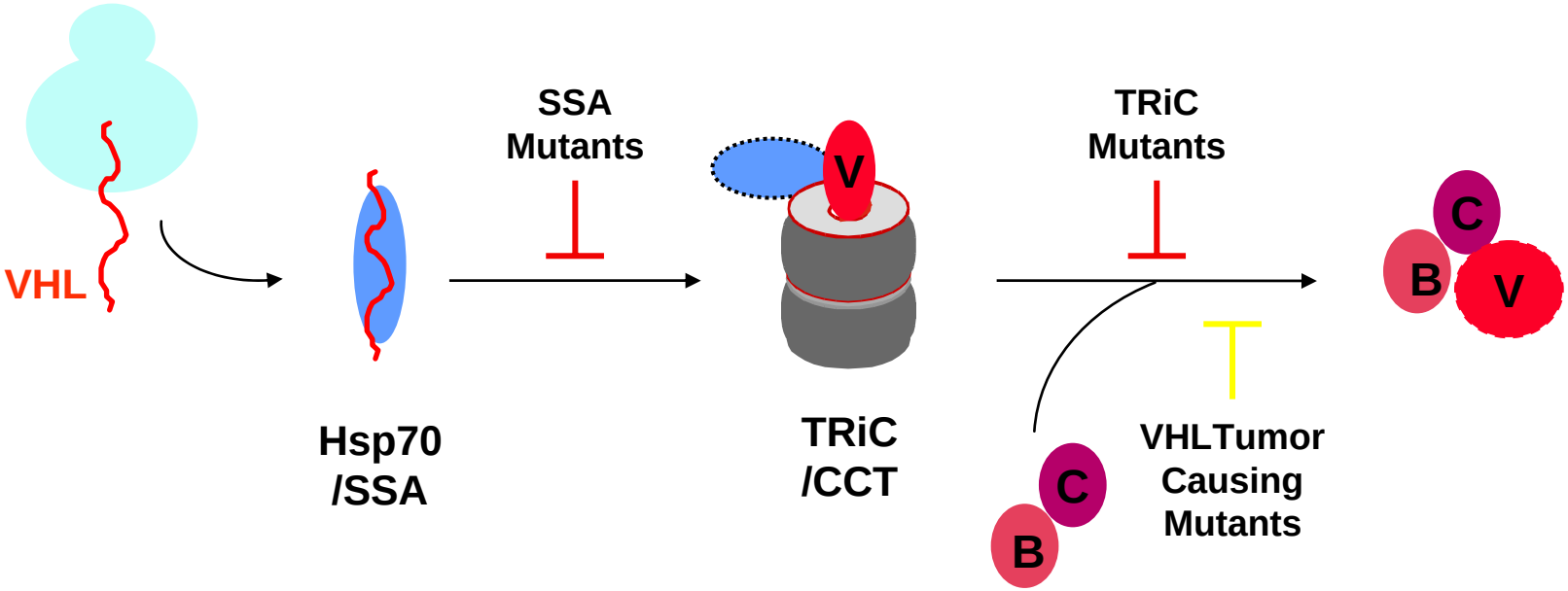
TRiC
~ 10%

Other Chaperones?
Unassisted?

EMBO J (1999)18, p.85

A Large Fraction of Cellular Proteins Transits Through Chaperones During their Biogenesis

Chaperones Cooperate During Protein Folding



Folding of the Tumor Suppressor VHL Requires the Cooperation of Hsp70 and TRiC/CCT

Molecular Cell (1999) 4, p.1051; Mol. Cell. Bio. (2003) 23, p.3141

Defects in Protein Folding lead to Human Disease

Amyloid Deposits: Prions, Alzheimers

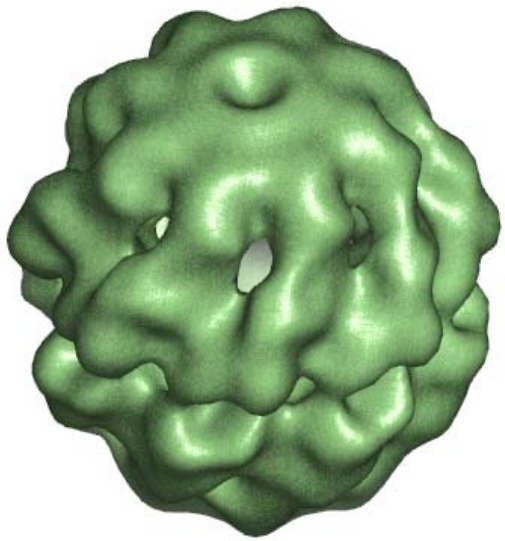
Mutations: Cancer, Metabolic Diseases

Denaturing Stress: Ischemia, Stroke

Chaperonin as a Model System

TRiC/CCT

an Eukaryotic chaperonin
made up of 8 distinct subunits



Mm-cpn

Archael chaperonin
made up of 8 identical subunits

- 16 subunits arranged in two rings
- Central cavity binds and folds substrate
- Built-in lid that opens and closes with ATP

Goals

- Engineer TRiC or Mm-cpn variants optimized to fold proteins of biomedical importance *in vitro.*
- Engineer TRiC or Mm-cpn variants that promote folding/unfolding of specific proteins *in vivo.*
- Develop the “adaptor” molecule approach to turn “on” or “off” selected proteins by targeting them to the chaperonin and eventually to other chaperones or the ubiquitin-proteasome system.
- Develop a versatile nano-container based on the chaperonin platform to encapsulate and release a number of different ligands.
- Develop and disseminate a pipeline for the quantitative characterization of nanomachines.

Four Engineering Questions About TRiC

- How does it open and close the cavity?
- What is the function of its subunit heterogeneity?
- How does it bind substrates?
- How does it fold substrates?

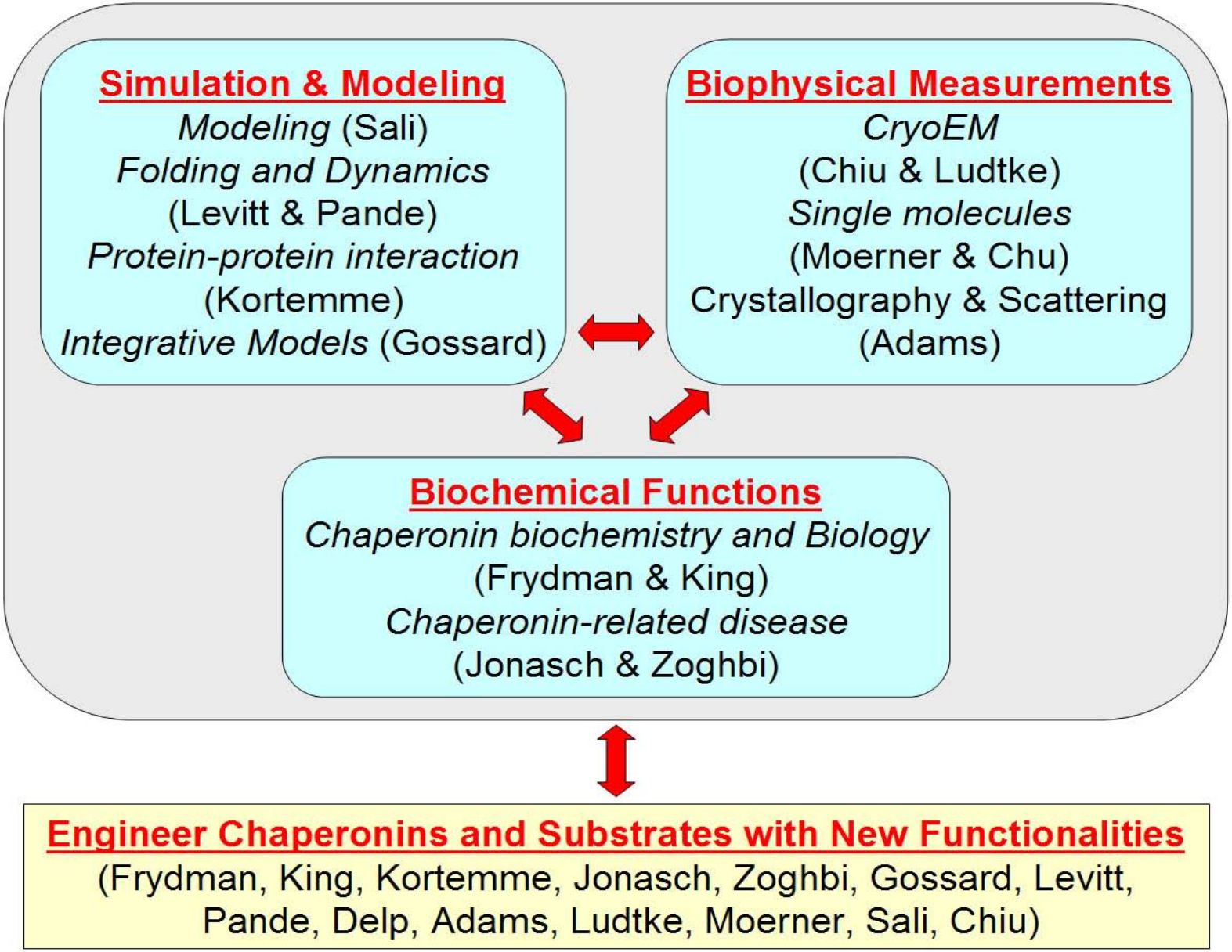
Knowledge Gaps

- Detailed chaperonin architectural design principles
- Proteomic analysis of substrates
- Chemical and physical basis for substrates and chaperonin interactions
- Chaperonin dynamics in vitro and in vivo
- Cellular networking of chaperonin and other cellular machines

Plausible Deliverables

- Structure signatures at different functional states
- Chemical characteristics
- Folding determinant factors
- Physical mechanism of folded protein release
- In silico prediction of modified chaperonin or substrate
- Annotation of chaperonin as a nano-device
- “System biology” of chaperonin *in vivo*

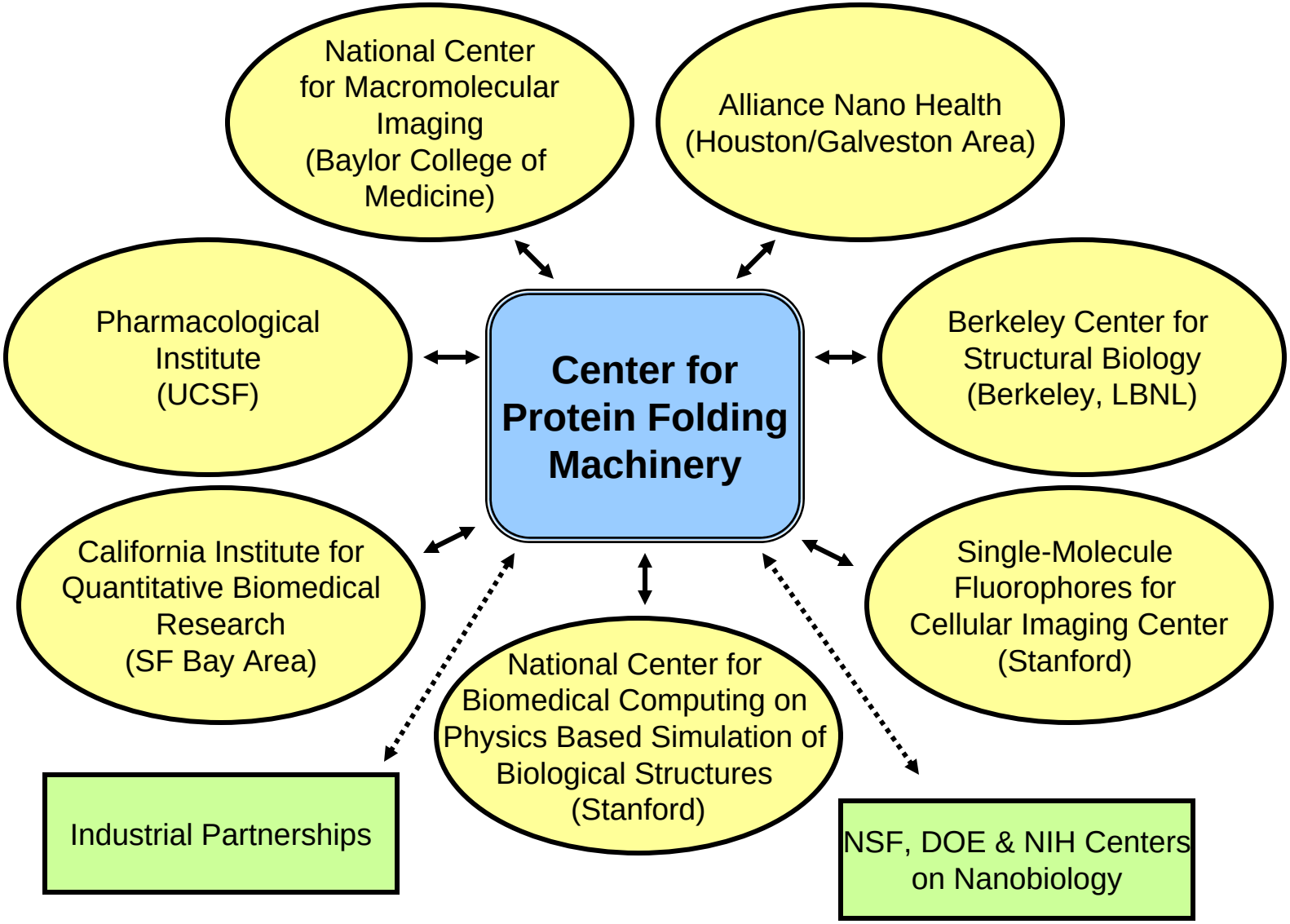
Integrated Approaches



Rationales of the Approaches

- Define engineering specifications of the chapernonin
- A pipeline of physical, chemical and computational assays of the engineered chapernonin
- Cyclic iterations between engineering specifications and functions of new products

Interactions with Other Centers



Training

- Undergraduate research training program in nanobiology

- Didactic course jointly taught by Center faculty across institutions